CLAIMS

1. A method for detecting a copy number of a specific nucleic acid per single cell in a cell population, the method comprising:

amplifying, in a reaction compartment of a plurality of reaction compartments each containing a Polymerase Chain Reaction (PCR) system and a DNA sample that is derived from a nucleic acid in a single cell, a target contained in the DNA sample by PCR; and

quantifying the copy number of the specific nucleic acid by quantifying an amplicon obtained by the PCR, the quantifying the amplicon being performed by measuring an intensity of fluorescence in each of the plurality of reaction compartments during an exponential amplification phase.

2. The method according to claim 1, wherein

the quantifying the amplicon includes

stopping a cycle of the PCR during the exponential amplification phase and measuring the intensity of the fluorescence in each of the plurality of reaction compartments, and

quantifying the amplicon obtained by the PCR from the intensity of the fluorescence.

3. The method according to claim 1 or 2, wherein the DNA sample is a set of genomic DNA in the single cell, a reverse transcription product of RNA in the single cell, or mitochondrial DNA in the single cell.

4. The method according to any one of claims 1 to 3, wherein the amplicon is quantified by a fluorescent probe method.

5. The method according to claim 4, wherein:

the PCR reaction system contains a plurality of probes respectively including

fluorescent materials with fluorescence wavelengths different from each other, the plurality of probes being respectively assigned to regions different from each other on the DNA sample;

each of the regions contains the target or a plurality of the targets; and

in the quantifying the amplicon, by measuring an intensity of fluorescence of a plurality of wavelengths from each of the plurality of reaction compartments, the reaction compartment in which the target or the plurality of targets contained in the DNA sample are amplified is distinguished from the reaction compartment in which only a contaminating cell-free nucleic acid is amplified.

6. The method according to claim 5, wherein:

each of the regions contains 5 to 100 of the targets to which a plurality of probes including fluorescent materials with fluorescence wavelengths identical to each other are assigned, respectively; and

in the quantifying the amplicon, for each of the regions, the amplicon is quantified by collectively measuring an intensity of fluorescence from the plurality of probes, regardless of a difference between the targets.

7. The method according to claim 5 or 6, wherein:

the DNA sample contains a set of genomic DNA and mitochondrial DNA both in the single cell;

the PCR reaction system contains a first probe including a fluorescent material with a first fluorescence wavelength and a second probe including a fluorescent material with a second fluorescence wavelength, the first probe being assigned to a region on the genomic DNA, the second probe being assigned to the mitochondrial DNA;

the region contains the target or the plurality of targets;

in the amplifying by the PCR, in each of the plurality of reaction compartments, the target or the plurality of targets contained in the set of genomic DNA and a target contained in the mitochondrial DNA are amplified by the PCR;

in the quantifying the amplicon, in each of the plurality of reaction compartments, the amplicon of the genomic DNA and the amplicon of mitochondrial DNA are quantified in a cycle during which amplification of the target contained in the mitochondrial DNA reaches a plateau and amplification of the target or the plurality of targets contained in the genomic DNA reaches the exponential amplification phase; and

in the quantifying the amplicon, by measuring an intensity of fluorescence of the first fluorescence wavelength and the second fluorescence wavelength from each of the plurality of reaction compartments, the reaction compartment in which the target or the plurality of targets contained in the genomic DNA and the target contained in the mitochondrial DNA are amplified is distinguished from the reaction compartment in which only a contaminating cell-free nucleic acid is amplified.

8. The method according to any one of claims 1 to 7, further comprising, generating the reaction compartment by lysing the single cell in a compartment containing the single cell, a cell lysis reagent, and a PCR premix.

9. The method according to any one of claims 1 to 8, further comprising:

lysing the single cell in a compartment containing the single cell; and

generating the reaction compartment by combining the compartment containing the single cell lysed with a compartment containing a PCR premix.

10. The method according to any one of claims 1 to 9, further comprising:

mixing a population of cell nuclei and a PCR premix in bulk; and

generating a plurality of the reaction compartments by separating the cell nuclei in the population of the cell nuclei together with the PCR premix from each other.

11. The method according to any one of claims 1 to 10, wherein the reaction compartment is a reaction droplet that is a droplet containing the DNA sample and the PCR reaction system.

12. The method according to any one of claims 1 to 11, wherein:

in the quantifying the copy number of the specific nucleic acid, the copy number of the specific nucleic acid is quantified by using a cutoff value as an external standard; and

the cutoff value is set in advance based on a result of the quantifying the amplicon obtained by the PCR for a cell in which the copy number of the specific nucleic acid is known.

13. The method according to claim 12, wherein before the result of the quantifying the amplicon obtained by the PCR is used to set the cutoff value, the result is corrected by using a result of a quantifying for a negative cluster.

14. The method according to any one of claims 1 to 13, wherein:

the DNA sample contains a set of genomic DNA in the single cell; and

the method further comprises detecting, from a result of the quantifying the amplicon, a presence of the single cell with at least one member selected from the group consisting of aneuploidy over an entire length of a chromosome, partial aneuploidy of a chromosome, gene amplification, and gene deletion.

15. The method according to claim 14, wherein the single cell contains the genomic DNA at least with a chromosomal mutation selected from below:

- aneuploidy of chromosome 21,

- aneuploidy of chromosome 18,
- aneuploidy of chromosome 13,
- aneuploidy of Y chromosome,
- aneuploidy of X chromosome,
- deletion of the 22q11.2 region on a long arm of chromosome 22,
- deletion of the 5q region on a short arm of chromosome 5,
- deletion of the 15q11-q13 region on a long arm of chromosome 15,
- amplification of a long arm of chromosome 1,
- deletion of a short arm of chromosome 17,
- deletion of a long arm of chromosome 13,
- deletion of a long arm of chromosome 4,
- deletion of a long arm of chromosome 5,
- deletion of a long arm of chromosome 7,
- amplification of chromosome 8,
- deletion of chromosome 11,
- aneuploidy of chromosome 12,
- deletion of a long arm of chromosome 20,
- deletion of a long arm of chromosome 19,
- deletion of chromosome 1,
- deletion of a long arm of chromosome 18,
- deletion of a short arm of chromosome 8,
- deletion of chromosome 4,
- amplification of a long arm of chromosome 8,
- deletion of a long arm of chromosome 16,
- amplification of a short arm of chromosome 5,
- amplification of a long arm of chromosome 3,

- deletion of a short arm of chromosome 3,
- deletion of a short arm of chromosome 9,
- gene amplification of MYCN gene,
- gene amplification of HER2 gene, and
- gene amplification of MET gene.

16. The method according to claim 14, further comprising:

generating data that includes information on whether or not the presence of the single cell is detected, wherein

the cell population is isolated from amniotic fluid or maternal blood so as to contain a fetal cell; and

the data is provided for a diagnosis of trisomy 13, trisomy 18, trisomy 21, Turner syndrome, triple X syndrome, XYY syndrome, Klinefelter syndrome, Di George syndrome, Angelman syndrome, Prader-Willi syndrome, or cri-du-chat syndrome.

17. The method according to claim 14, further comprising:

generating data that includes information on whether or not the presence of the single cell is detected, wherein

the cell population is isolated from a patient, and

the data is provided for a diagnosis of myelodysplastic syndrome, multiple myeloma, idiopathic eosinophilia, chronic eosinophilic leukemia, acute nonlymphocytic leukemia, myeloproliferative neoplasm, chronic lymphocytic leukemia, acute myeloid leukemia, brain tumor, neuroblastoma, colon cancer, breast cancer, ovarian cancer, cervical cancer, endometrial cancer, small cell lung cancer, non-small cell lung cancer, bladder cancer, kidney cancer, liver cancer, pancreatic cancer, esophageal cancer, thyroid cancer, or head and neck cancer.